

Application No. 10/784,163
Reply to Office Action of October 18, 2007
and the Communication dated December 17, 2008

IN THE CLAIMS

Please amend the claims as follows:

Claims 1-115 (Cancelled).

116. (New) A method of detecting two or more antigens in a sample, comprising simultaneously contacting a sample, which has been previously simultaneously contacted with a primary antibody cocktail comprising at least one first primary antibody and at least one second primary antibody in a buffered aqueous solution suitable to stabilize the primary antibody cocktail, with a composition comprising at least one first secondary antibody and at least one second secondary antibody to form at least two antigen-antibody complexes on the sample, wherein the at least one first secondary antibody is coupled to a poly (alkaline phosphatase) moiety and the at least one second secondary antibody is coupled to a poly (horseradish peroxidase) moiety, and wherein the composition comprises a buffer suitable to stabilize the first and second secondary antibodies; and detecting the at least two antigen-antibody complexes on the sample, wherein the detection of the antigen-antibody complexes is more sensitive when compared to a process where the secondary antibodies are ALP- or HRP-conjugated secondary antibodies and wherein the method can be completed in not more than 2 to 2.5 hours.

117. (New) The method of claim 116, wherein
the at least one first secondary antibody and the at least one second secondary antibody is a goat anti-mouse antibody or goat anti rabbit antibody; and
another of the at least one first secondary antibody and the at least one second secondary antibody is a goat-anti-rabbit antibody or goat anti mouse antibody.

118. (New) The method of claim 116, wherein the detecting comprises applying DAB and Fast Red to the sample.

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119. (New) The method of claim 116, which comprises detecting at least three antigens in a sample wherein the primary antibody cocktail further comprises at least a third primary antibody; and the method further comprises simultaneously contacting the sample with at least one third secondary antibody to form at least one third antigen-antibody complexes on the sample, and detecting the at least one third antibody-antigen complex on the sample.

120. (New) The method of claim 119, which comprises detecting at least four antigens in a sample wherein the primary antibody cocktail further comprises at least a fourth primary antibody; and the method further comprises simultaneously contacting the sample with the conjugated poly-HRP and/or poly-AP secondary antibody to form at least one fourth antigen-antibody complexes on the sample detecting the at least one fourth antibody-antigen complex on the sample.

121 (New) The method of claim 116, wherein the sample is contained in an automated staining device and wherein the contacting occurs in the automated staining device.

122. (New) The method of claim 116, wherein at least one of the at least one first primary antibody and the at least one second primary antibody is a rabbit monoclonal antibody.

123. (New) The method of claim 120, which comprises detecting at least five or six antigens in a sample and wherein the primary antibody cocktail further comprises at least a 5 or 6 primary antibodies; and wherein the method further comprises simultaneously contacting the sample with at least one fifth and/or sixth secondary antibodies to form at least one fifth and/or sixth antigen-antibody complexes on the sample, and detecting the at least one fifth and/or sixth antibody-antigen complexes on the sample.

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124 (New) The method of claim 116, wherein the process from application of the primary antibody cocktail to detecting the antigen-antibody complexes is completed in not more than 15 steps including washing steps.

125 (New) A method of detecting two or more primary antibodies including one or more mouse and one or more rabbit antibodies in a stabilized diluent that have been cocktailed and applied simultaneously on a tissue/cell sample, the method comprising simultaneously detecting the primary antibody cocktail that has coupled with a specific antigen by applying a secondary antibody cocktail of a conjugated goat anti-mouse or rabbit poly-horseradish peroxidase enzyme (HRP); or a goat anti-mouse or rabbit poly-alkaline phosphastase enzyme (AP) in a buffer suitable to stabilize the secondary antibody cocktail; and

applying DAB (HRP) and Fast Red to the sample to visualize a chromogenic reaction comprising of the simultaneous primary antibody cocktail and the simultaneous detection cocktail of HRP and AP,

wherein the process from application of the primary antibody cocktail to detecting the antigen-antibody complexes and visualizing with a chromogen is completed in not more than 15 steps including washing steps, wherein the detection of the antigen-antibody complexes is more sensitive when compared to a process where the secondary antibodies are ALP- or HRP-conjugated secondary antibodies, and wherein the method can be completed in less than 2 hours on a automated platform.

126. (New) A method of detecting two or more antigens in a sample, comprising simultaneously contacting a sample with a primary antibody cocktail comprising at least one first primary antibody, and at least one second primary antibody in a buffered aqueous

solution suitable to stabilize the primary antibody cocktail, subsequently simultaneously contacting the sample with a composition comprising at least one first secondary antibody and at least one second secondary antibody to form at least two antigen-antibody complexes on the sample, wherein the at least one first secondary antibody is coupled to a poly (alkaline phosphatase) moiety and the at least one second secondary antibody is coupled to a poly (horseradish peroxidase) moiety; and detecting the at least two antigen-antibody complexes on the sample, wherein the detection of the antigen-antibody complexes is more sensitive when compared to a process where the secondary antibodies are ALP- or HRP-conjugated secondary antibodies and wherein the method can be completed in less than 2 hours.

127. (New) The method of claim 126, wherein the at least one first secondary antibody and at least one second secondary antibody, which is a goat anti-mouse antibody or goat anti rabbit antibody, and another of the at least one first secondary antibody and at least one second secondary antibody which is a goat-anti-rabbit antibody or goat anti mouse antibody.

128. (New) The method of claim 126, wherein the detecting comprises applying DAB and Fast Red consecutively.

129. (New) The method of claim 126, which comprises detecting at least three antigens in a sample wherein the primary antibody cocktail further comprises of at least a third primary antibody; and the method further comprises simultaneously contacting the sample with at least one third primary antibody cocktail with at least one second secondary antibody, which is a goat anti-mouse antibody or goat anti rabbit antibody, and another of at least one third primary antibody cocktail and at least one second secondary antibody, which is a goat-anti-rabbit antibody or goat anti mouse antibody, and detecting at least one third antibody-antigen complex on the sample.

130. (New) The method of claim 129, which comprises detecting at least four antigens in a sample wherein the primary antibody cocktail further comprises at least a fourth primary antibody; and the method further comprises simultaneously contacting the sample with at least one fourth of primary antibody cocktail and one second secondary antibody, which is a goat anti-mouse antibody or goat anti rabbit antibody, and another of the at least one second secondary antibody, which is a goat-anti-rabbit antibody or goat anti mouse antibody, and

detecting the at least one fourth antibody-antigen complex on the sample.

131 (New) The method of claim 126, wherein the sample is contained in an automated staining device and wherein the contacting occurs in the automated staining device.

132. (New) The method of claim 130, wherein at least one of the first or one of the second primary antibodies is a rabbit monoclonal antibody.

133. (New) The method of claim 130, which comprises detecting at least five or six antigens in a sample and wherein the primary antibody cocktail further comprises at least a 5 or 6 primary antibodies; and wherein the method further comprises simultaneously contacting the sample with at least one fifth and/or sixth primary antibody cocktail and at least one fifth and/or sixth secondary antibody to form at least one fifth and/or sixth antigen-antibody complexes on the sample, and detecting the at least one fifth and/or sixth antibody-antigen complexes on the sample.

134 (New) The method of claim 126, wherein the process from application of the primary antibody cocktail to detecting the antigen-antibody complexes is completed in not more than 15 steps including washing steps.

135. (New) The method of claim 116, wherein the buffered aqueous solution stabilizing the primary antibody cocktail comprises 2-methyl-4-isothiazolin-3-one as a preservative.

136. (New) The method of claim 125, wherein the buffered aqueous solution stabilizing the primary antibody cocktail comprises 2-methyl-4-isothiazolin-3-one as a preservative.

137. (New) The method of claim 126, wherein the buffered aqueous solution stabilizing the primary antibody cocktail comprises 2-methyl-4-isothiazolin-3-one as a preservative.